GROUPING AND MUTATION IN BOTRYCHIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 273

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(WITH ELEVEN FIGURES)

Ever since the appearance of CHRYSLER'S[1] paper, claiming that the fertile spike of *Botrychium* represents a pair of fused pinnae of the vegetative leaf, I have been interested to note the peculiarities in the spore-bearing portions, upon which he relies for a part of his evidence. Two fertile spikes in the position of the lower pair of leaflets, spikes in which the two component leaflets are incompletely fused, sporangia on the second pair of leaflets in addition to the sporangia of the fertile spike, and occasional sporangia on vegetative leaves were found during vacation field studies of *Botrychium obliquum* and its varieties. CHRYSLER'S claim needs no support, his evidence both from field study and anatomy being so convincing that, for years, we have treated these forms as a mere family, Ophioglossaceae, under the Filicales.

However, the field studies, carried on for several years during September vacations in Ohio, at Oberlin, Sullivan, Cleveland, and Birmingham, together with a few observations at Osborn, Indiana, and at Fort Sheridan, Illinois, impressed upon me that one scarcely ever finds isolated plants of *Botrychium*. They are almost invariably grouped; even when there seems to be an isolated plant, others can usually be found in the immediate vicinity.

In *Ophioglossum* there is abundant vegetative propagation by branching of the rhizome, so that not only are the plants grouped, but the plants of a group are more or less connected. In striking contrast, *Botrychium* shows no vegetative propagation. The rhizome scarcely ever branches and, when it does, the branch is not likely to become separated and form a new plant. Every plant comes from a prothallium. Consequently the distribution is

[1] CHRYSLER, M. A., The nature of the fertile spike in Ophioglossaceae. Ann. Botany 24: 1–18. 1910.

entirely by spores; and since the prothallia are of the subterranean tuberous type, with an endophytic fungus, the prothallia develop only when the conditions for this rather unusual mode of development are present.

How far the spores might be carried is problematical. The grouping of plants indicates that most of the spores are not carried far, but when a plant is once established it becomes the center of a group.

At first I was interested only in the fact of grouping and in the size of the groups of *Botrychium obliquum* and *B. virginianum.* It was noted immediately that the groups of *B. virginianum* contained many more plants than those of *B. obliquum*, and that the groups were more closely associated. In counting plants and making plots, one soon learns to find specimens, especially the smaller ones, which easily escape notice, and the number of plants in a group is likely to be surprisingly larger than the average botanist would have guessed from a cursory examination.

The most closely associated groups, with the largest number of plants in a group, were found at the borders of rather open woods. Plants in the deeper woods, although likely to be large and vigorous, are not abundant.

During the Septembers of the past four years the grouping was observed, and a searching for prothallia developed some facility in recognizing young plants. In 1918 the plants of many groups were counted, especially at Sullivan, where *Botrychium* is exceptionally abundant; and in 1919 plots were made, showing not only the number and position of plants in a group, but also the relation of the groups to each other.

Botrychium virginianum is more abundant than *B. obliquum*, even when the two species are growing together under the same conditions. On the eastern border of a densely wooded tract at Sullivan, Ohio, where *B. virginianum* is more abundant than I have ever seen it in any other locality, prothallia were collected and observations were made for several years. The border of the woods is roughly marked by a rail fence, with but few trees on the eastern side and some trees removed on the western side, so that the woods end in what farmers call a "clearing." The plants and prothallia are most abundant in the clearing, within 25 m. of the fence, becoming more and more scattered as the woods become denser, while at a distance of 200 m. west of the fence scarcely any plants are found. In this place plants are most abundant on little elevations caused by uprooted trees. When a large tree is blown down, the roots tear up a considerable quantity of soil, so that when the tree decays and disappears there remains a mound with a depression on one side of it. These little mounds of clay soil, scantily covered by moldy humus, seem to be exceptionally favorable places for the germination of spores and the growth of plants.

A few years ago, before any plots were made, the abundance of plants in this locality suggested counting the number on definite areas. These areas do not correspond exactly to the groups which were plotted later, because only the denser centers of the groups were considered, the more scattered plants at the borders being omitted. Some of the highest countings of plants on given areas are worth recording. On areas of 1 sq. m. there were 15, 20, 29, 30, 31, 42, 66, and 106 plants. In the last case the plants were very closely crowded, one cluster of 5 plants occupying a space only 3 cm. sq. On areas of 2 sq. m., there were counted 27, 43, 70, and 103 plants; on 4 sq. m., 112 plants; on 2 dm. sq., 7, 10, and 16 plants; and on 2.3 dm. sq., 8 and 21 plants.

In this clearing the groups were rather closely associated, being separated from each other by distances of 1–10 m., with only here and there a plant between. Many of these plants were small, some of them sporelings still attached to prothallia; but in any place where *Botrychium* is abundant there will be a goodly number of large plants. In such places white patches of the fungus can be seen by turning over the leaf mold.

Aside from noting the grouping and counting the number of plants in a group, little was done with *B. virginianum*. The same must be said of *B. simplex*, which was discovered accidentally at Osborn, Indiana, during a search for *Ophioglossum*. A group of a dozen specimens of this little *Botrychium* was found on an area of about 1 sq. m. So far as we know, this species has not been reported for the Chicago region.

The principal interest centered in *B. obliquum* and *B. dissectum*, which is often regarded as a variety of *B. obliquum*. There are other forms which taxonomists describe as varieties of *B. obliquum* and which may be as distinct and may have as definite a relation to the parent form as we believe *B. dissectum* has to *B. obliquum;* but we did not make any study of these forms, and in making plots and in counting we recognized only *B. dissectum*, and put all the rest—the varieties *oneidense*, *tenuifolium*, and *elongatum*—under the general name *B. obliquum*. Besides these varieties, which can often be identified with a manual, there are fluctuating variations, so that one who is not a professional taxonomist is tempted to call the whole assemblage *B. obliquum* and let the name cover *B. obliquum* and its derivatives.

B. obliquum does not occur in such large numbers as *B. virginianum*, the plants of a group being more scattered, with seldom more than a dozen plants on 1 sq. m. This difference in numbers and the difference in grouping is indicated in fig. 1. This plot represents an area of 33 by 40 m. The dots and the crosses of the diagram are all of one size, but the plants varied from sporelings still attached to prothallia up to large specimens. Where plants are so numerous, as indicated in the denser groups, not more than a quarter of them have fruiting spikes.

Why plants of *B. virginianum* should be so much more numerous than those of *B. obliquum*, when they are growing in the same situation, particularly when growing on the same spot, as shown in the lower right hand group in fig. 1, is not obvious. A million spores would be a very conservative estimate for the output of an average plant of either species, and in the largest plants the output probably reaches five or six million spores; but a comparison of the number of plants and the number of spores would indicate that far less than one spore in a million produces a plant which can be seen above ground. One might guess that spores which do not sift down immediately to a safe depth die very soon from exposure or only a little later from the winter's cold; but we have noticed that in the tropical rainy forests of southern Mexico, where *Botrychium* is abundant and where there would seem to be no danger from dryness or cold, prothallia are as difficult to find as in the United

States. It may be possible that differences in the sculpturing on the spore coats may facilitate or impede the penetration of the

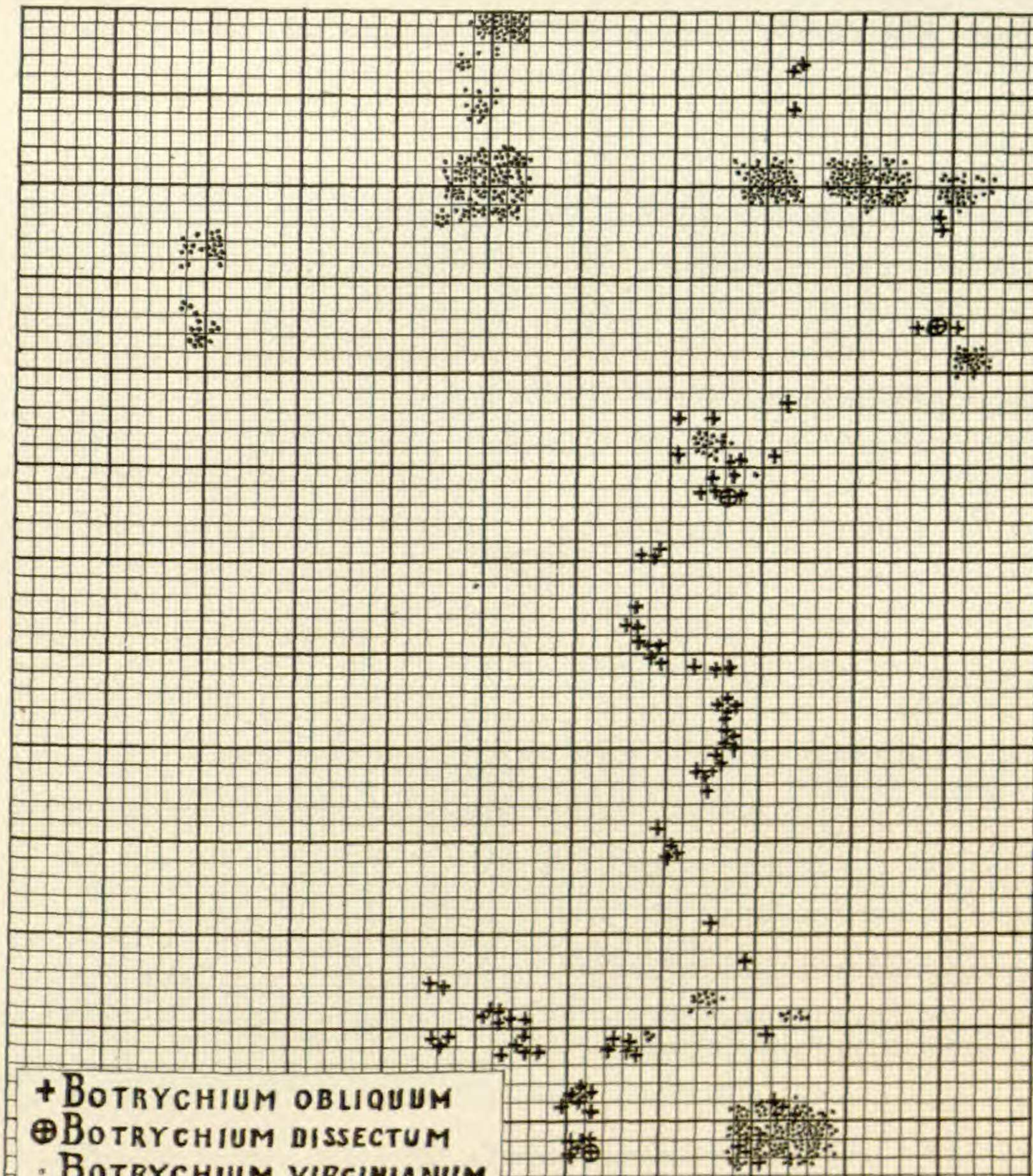


FIG. 1.—Plot 33×40 m. at Sullivan, Ohio, showing *Botrychium virginianum*, *B. obliquum*, and *B. dissectum;* each dot represents a plant of *B. virginianum;* each cross, a plant of *B. obliquum;* and each cross in a circle, a plant of *B. dissectum*.

spores to a favorable depth, or may favor or impede the absorption of water, and thus account for the larger number of plants of *B. virginianum*.

The principal study of *B. obliquum* was made at Oberlin, Ohio, in the cemetery, a part of which is sparsely covered by the original timber, while the rest is still more sparsely dotted with *Juniperus, Pinus, Thuja,* and *Cupressus.* Of the 24 groups which were counted

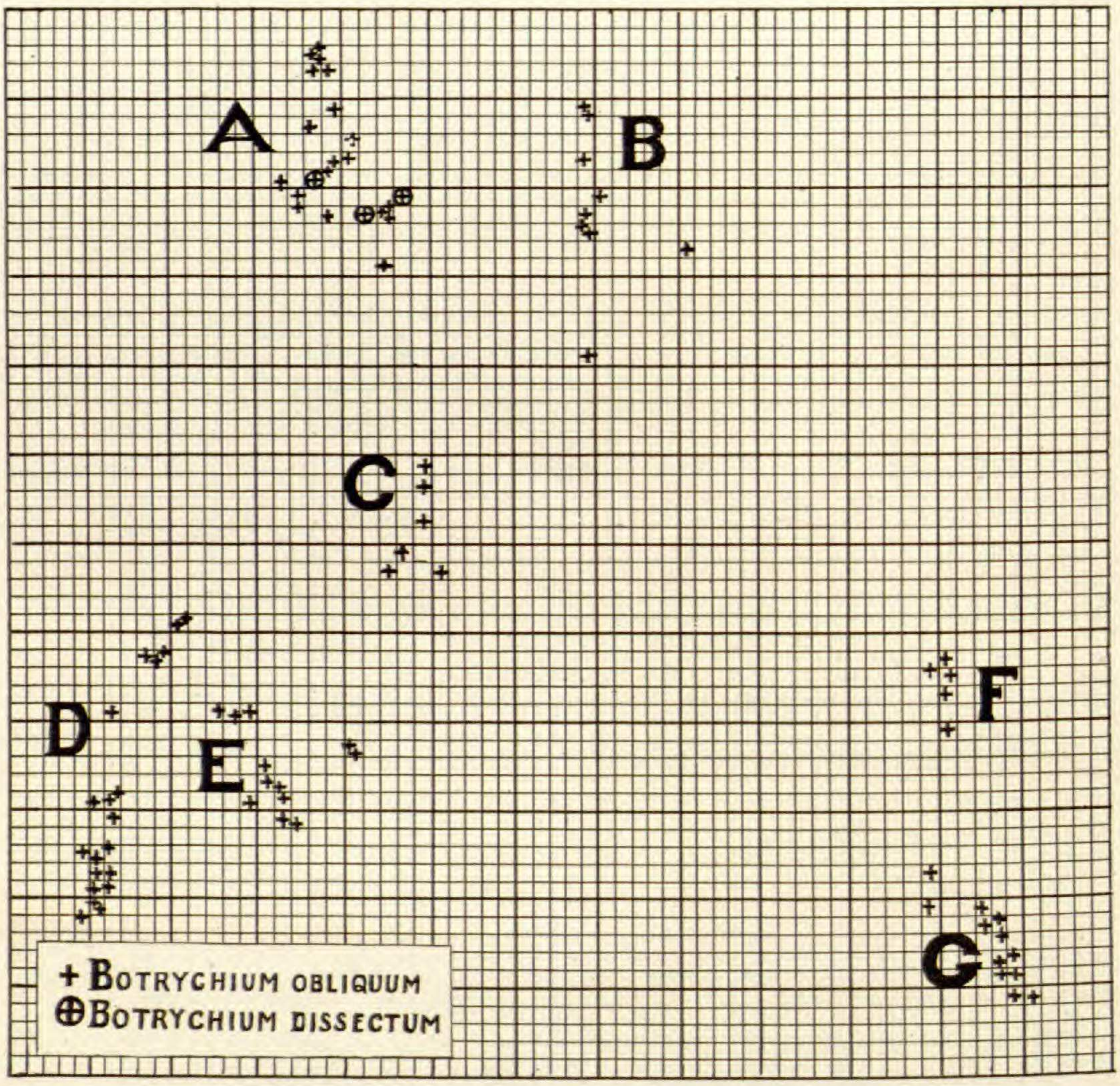


FIG. 2.—Plot about 40×43 m. at Oberlin, Ohio: distances between individual plants of a group approximately correct, but distances between groups *A* and *C*, *C* and *D*, *C* and *F*, and *F* and *G* about twice as great as indicated; there is no *B. virginianum* in this vicinity.

and plotted, 17 were at this place, 4 at Sullivan, 2 at Cleveland, and 1 at Pittsfield. A sample of the plotting at Oberlin is given in fig. 2.

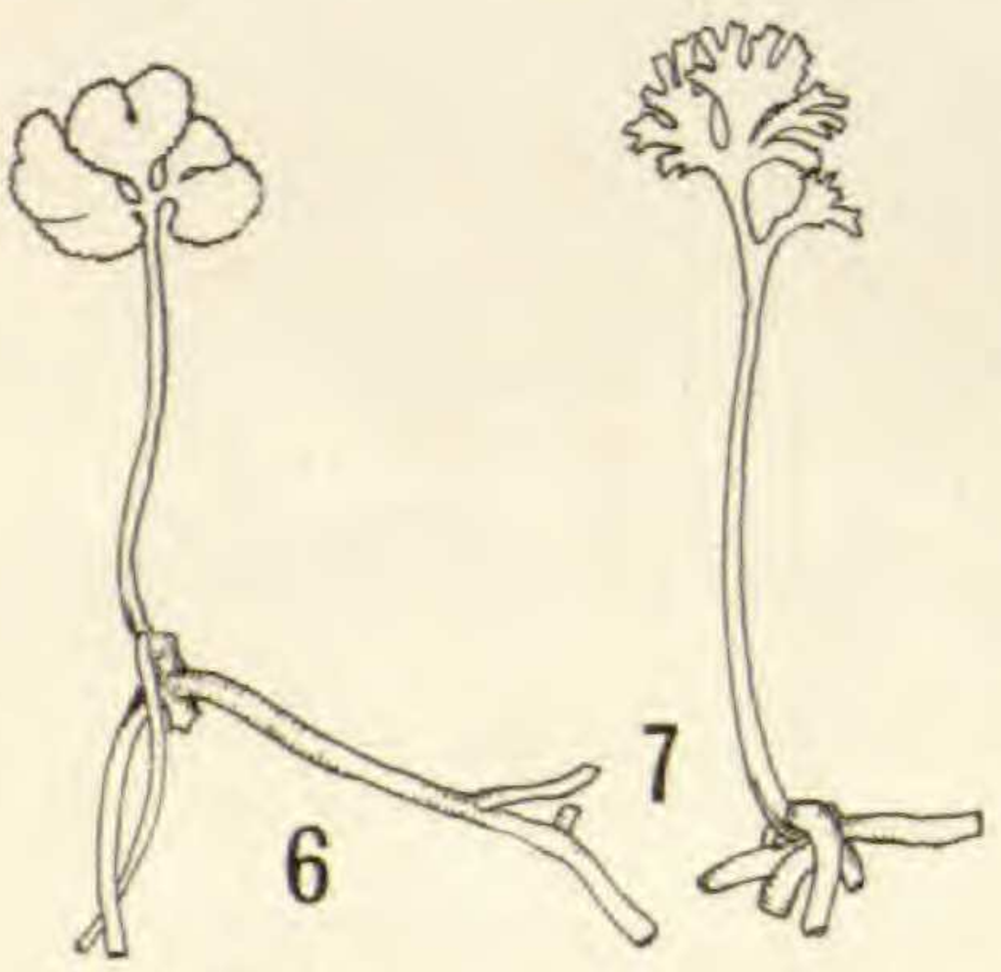
It was from such detailed field studies as those shown in figs. 1 and 2 that we reached the conclusion that *B. dissectum* is a mutant from *B. obliquum*. In ordinary cases such a conclusion would be tested by sowing the spores and growing the plants; but, so far as we are aware, no one has ever succeeded in raising prothallia of any species of *Botrychium* from the spore. Even if someone should find out how to grow prothallia and sporelings, it would

FIGS. 3–5.—Two vigorous plants of *B. obliquum*; plant of *B. dissectum* with typical leaf and roots, but with unusually good sporangia: small plant of *B. obliquum*.

take a long time to secure any results. How long the spore may rest before germinating is problematical; but even after it germinates it is probably a year or more before the prothallium reaches the fertilization stage. In adult plants the leaf is in its fourth year when it appears above ground. Consequently at least five years, and more probably six or eight years, would elapse between the sowing of the spores and the appearance above ground of the first tiny leaf of the sporeling. We have seen one sporeling with a fertile spike bearing a few sporangia while still attached to the prothallium, but it is probable that ten or twelve years usually elapse between the germination of the spore and the development of a plant up to the spore-bearing stage. During the past twenty years we have made so many unsuccessful attempts to germinate *Botrychium* that we did not even try to test our theory by this method, but collected some circumstantial evidence which supports our conclusion that *B. dissectum* is a mutant from *B. obliquum.*

Before presenting the evidence it is worth while to call attention to the distinguishing characters of the two forms.

B. obliquum and its varieties have oblique leaflets with margins ranging from nearly entire to quite sharply serrate, sometimes doubly serrate, while *B. dissectum* has a leaflet, still oblique in topography, but so dissected that the specific name is very appropriate (figs. 3–5). This difference in leaves is recognizable even in the sporeling (figs. 6 and 7). The leaves of sporelings are simpler in outline than those of larger plants, but the general character of the margins is characteristic from the first, so that there is no danger of confusing the forms.



FIGS. 6, 7.—Sporeling of *B. obliquum;* natural size; sporeling of *B. dissectum;* natural size.

In *B. obliquum* and its varieties there is considerable variation in the shape of the leaflet and in the character of the margin; but, so far as the margin is concerned, the differences are confined to a greater or lesser degree of serration. The deepest serration of *B. obliquum* would not be mistaken for the deeply cut margins of *B. dissectum.* In *B. dissectum* there is also some variation in the margins, but the dissected character is always evident, the differences being in the extent of the dissection (fig. 8).

We are familiar, of course, with the great variations in the leaflets of cultivated ferns, where a single leaf may have leaflets with a nearly entire margin, leaflets deeply cut, and still others so deeply cut that the bipinnate condition is reached. We do not regard *Botrychium* as a similar case, but believe that the differences in the margins of *B. obliquum* and *B. dissectum* are more like the differences in the margins of the leaflets of *Bowenia spectabilis* and *B. serrulata*, and like the differences in the leaf margins of *Dioon edule* and *D. spinulosum*. In these four cycads, the margins are so constant that they are reliable diagnostic characters.

The short subterranean stem, with the long-stalked leaf and spore-bearing portion with a still longer stalk, is similar in *B. obliquum* and *B. dissectum*.

The roots of *B. dissectum* are wrinkled and fleshy, like those of *B. obliquum*, and not at all like the slender roots figured in BRITTON and BROWN'S *Illustrated Flora*.

In general, *B. obliquum* is a larger plant than *B. dissectum*. At Oberlin the largest plant of *B. obliquum* measured 35 cm. in height, with a leaf 20 cm. in width, and the spore-bearing part of the fertile spike 15 cm. long. While this is not quite up to the limit in size recorded for the species, it is very large, and most individuals are much smaller. One plant of *B. dissectum* measured 28 cm. in height, but this is exceptional. The usual size of *B. dissectum* is about two-thirds that of *B. obliquum*.

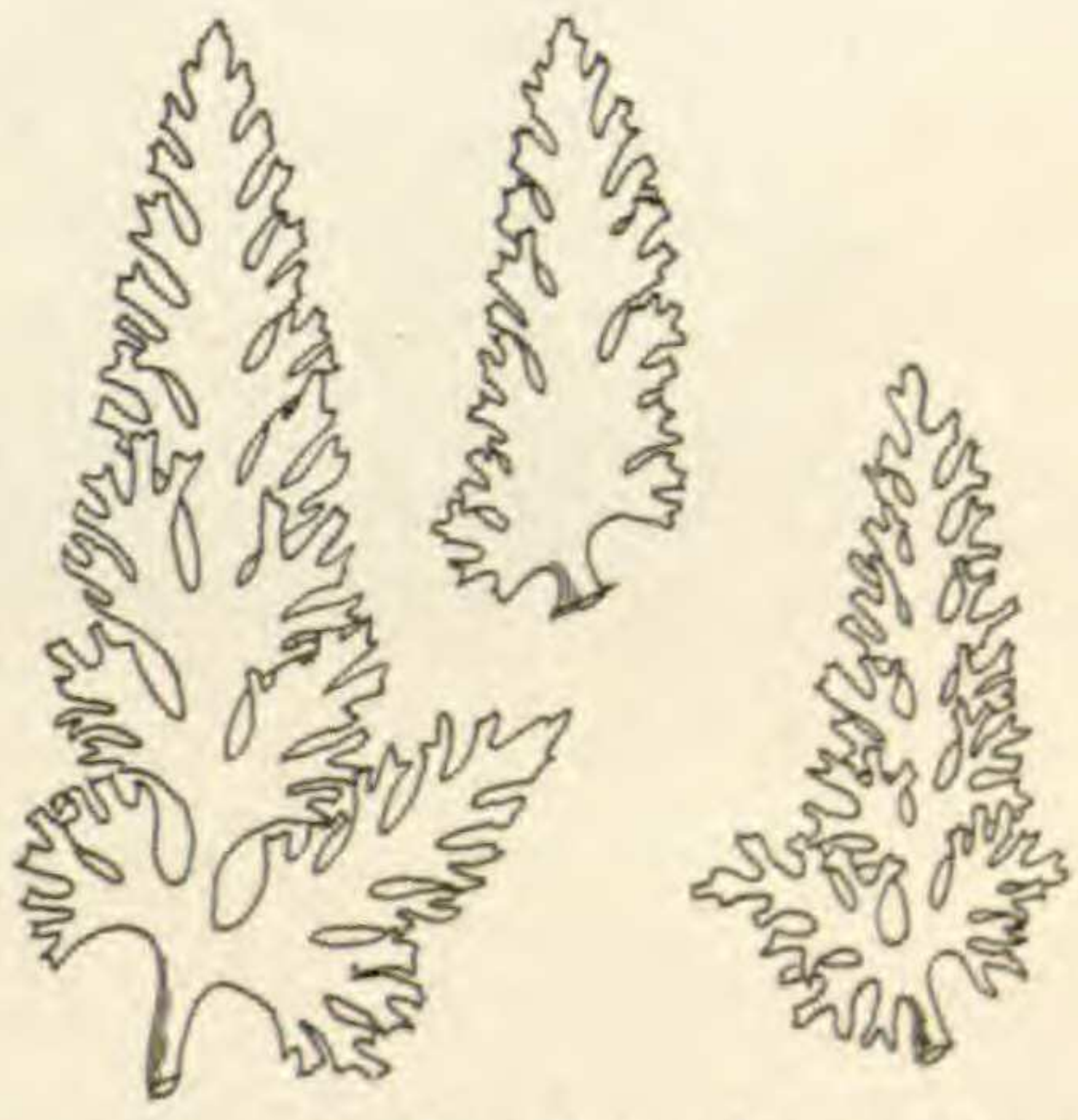
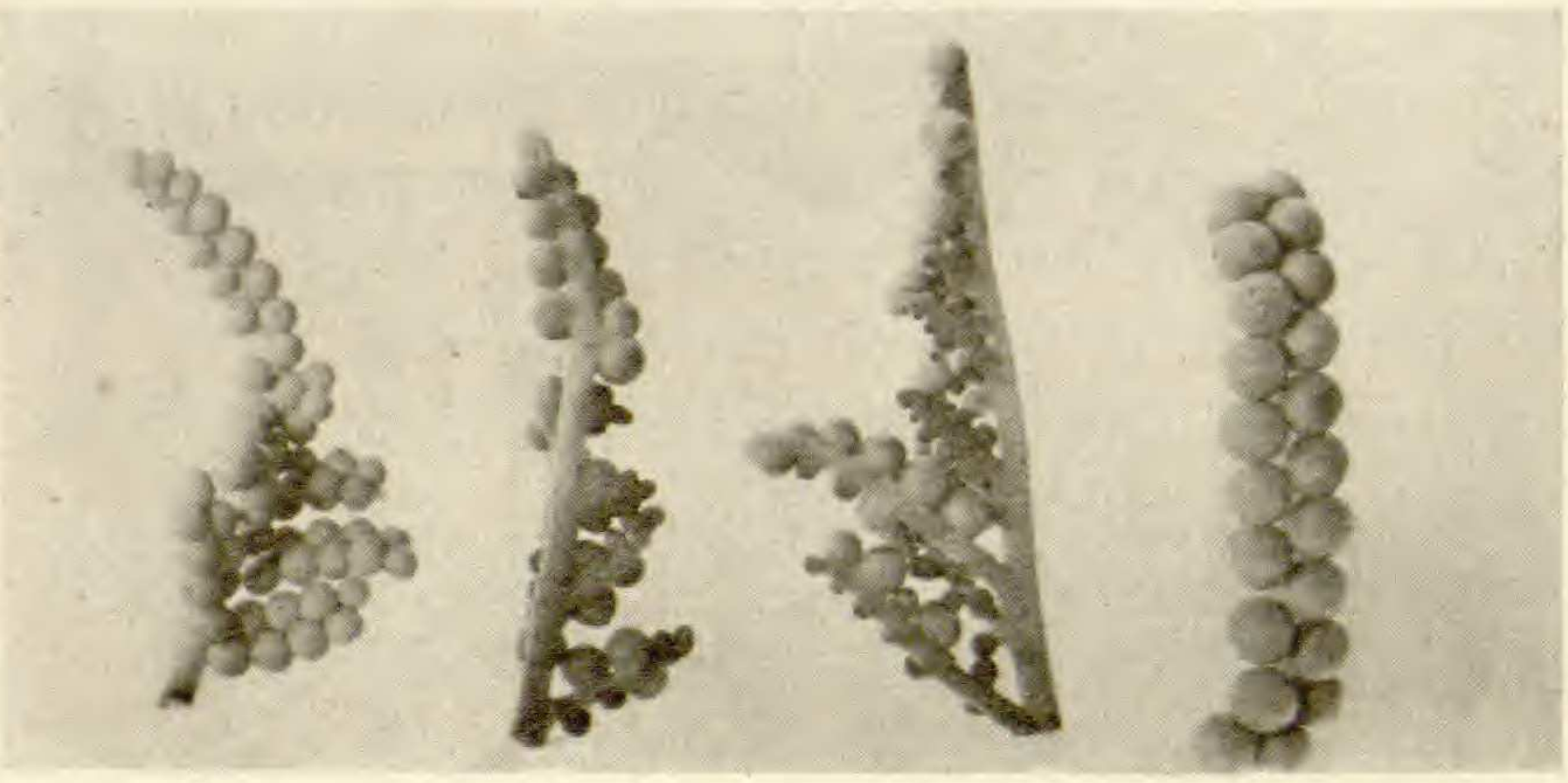


FIG. 8.—Leaflets from three plants of *B. dissectum*; natural size.

The most suggestive difference between *B. obliquum* and *B. dissectum* is seen in the fertile spike. The sporangia of *B. dissectum* sometimes look uniform and perfect, but somewhat smaller than those of *B. obliquum*. The difference in size, where the sporangia seem to be perfect, may be seen by comparing *A* and *D* of fig. 9.

However, most specimens show a considerable proportion of abortive sporangia which, even without sectioning, may be distinguished by their smaller size (fig. 9, *B* and *C*). The figure of *B. dissectum* in BRITTON and BROWN'S *Illustrated Flora* is evidently drawn from a specimen with sporangia like those in fig. 9, *B* and *C*, and in this respect is characteristic.

Sections of sporangia, like those shown in fig. 9, *B* and *C*, show that the smaller sporangia contain no spores at all (fig. 10). In most of these cases the sporangium wall is from 4 to 6 cells thick, with the inner layer not differentiated into a definite tapetum, and the outer lacking the anticlinal thickenings so characteristic of sporangia which produce even imperfect spores (fig. 11). In extreme cases the sporangium is a mere mass of parenchyma cells; in others, a narrow streak of mucilage indicates that sporogenous tissue had begun to form; in still others, like the one shown in



A *B* *C* *D*

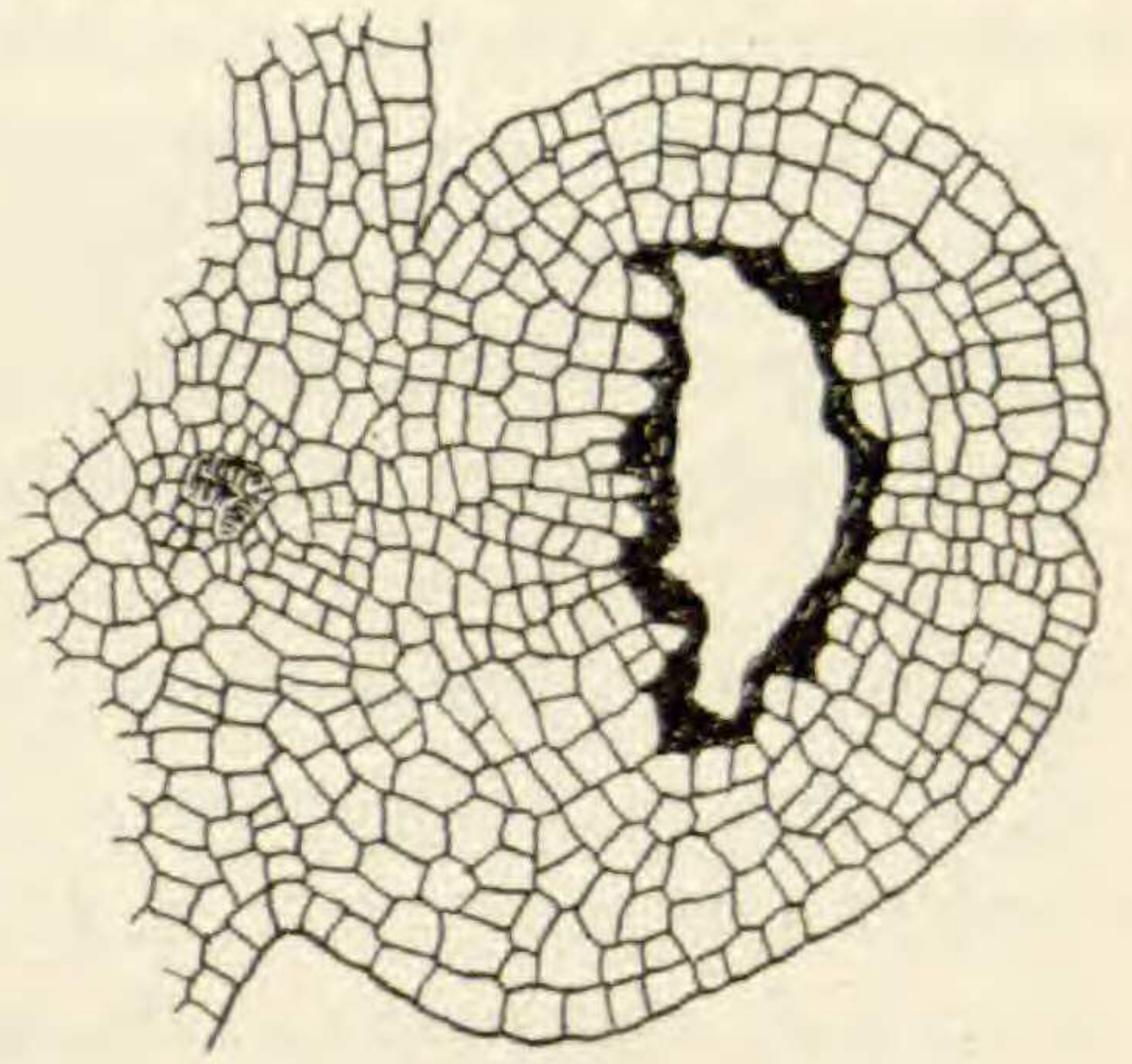
FIG. 9.—*A*, *B*, *C*, *B. dissectum*; *D*, *B. obliquum*; ×2

fig. 10, a considerable mass of sporogenous tissue has been formed; and in a few cases it could be seen that the mucilaginous mass consisted partly of imperfect, disorganizing spores.

In the apparently perfect sporangia of *B. dissectum* many of the spores are somewhat smaller than the average size for *B. obliquum*, and there are many spores which look as if they might be abortive. Fig. 11 shows six spores still floating in the tapetal plasmodium. The two spores at the upper left, one of them triangular in outline, are doubtless abortive; of the other four, only the one at the lower left has the full diameter of a normal spore of *B. obliquum*. The epidermal layer has anticlinal thickenings, as in normal sporangia, which dehisce and shed their spores.

It would be interesting to compare the reduction divisions of the two species, but the problem of getting material of *B. dissectum* makes such a comparison difficult, if not impossible. Even with good preparations of critical stages, the interpretation might be uncertain, for, judging by a few figures in *B. obliquum*, the $2x$ number is well over 100, and the chromosomes are tangled and hard to count.

Such evidence as we have would indicate that *B. dissectum* is at least partially, and probably entirely, sterile. Unfortunately the natural test which would prove or disprove this theory—germinating the spores—cannot be applied until someone learns how to make these baffling spores germinate. If the spores of *B. dissectum* germinate, we do not see why this species should not occur in groups, like *B. virginianum*, *B. obliquum*, *B. simplex*, and probably the other species.



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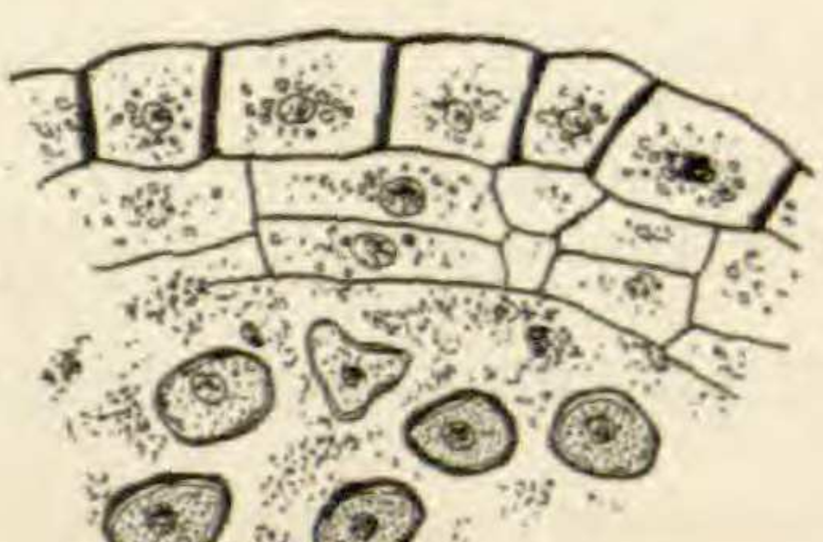

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FIGS. 10, 11.—Abortive sporangium of *B. dissectum*, with many-layered wall and mass of mucilage lining cavity from which sporogenous tissue has been resorbed; ×160: portion of sporangium of *B. dissectum*, showing spores of different sizes; triangular spore doubtless abortive; ×350.

In our opinion, the explanation of the occurrence and behavior of *B. dissectum* is that the species is a sterile mutant from *B. obliquum*. The principal facts supporting this theory are that *B. dissectum*, so far as I have observed in a five years' study, never occurs except in association with *B. obliquum*, and that there is no evidence that it reproduces itself.

It might be objected that mutants do not occur so frequently as *B. dissectum* would indicate, and it must be admitted that the total number of plants in our plots is not as large as one might wish in making ratios. The total number of plants in the twenty-four plots was 482 of *B. obliquum* and 19 of *B. dissectum*, a ratio of 25:1. The ratio in the Oberlin group was 20:1; in the Sullivan group, about 48:1; and in the Cleveland group, 40:1.

However, in making any objections to the theory on the ground that *B. dissectum* occurs too frequently to be a mutant, it must be remembered that mutation in plants has been studied almost exclusively in angiosperms, which are heterosporous and which have comparatively low chromosome numbers. We are assuming that mutations occur in the mitotic mechanism, probably during the reduction divisions, so that the mutant, which one recognizes in the sporeling stage, is merely the result of a preceding phenomenon.

At first thought, someone might suggest that *B. dissectum* is a hybrid. What species could cross to give such characters as we find in *B. dissectum?* The mere question seems a sufficient answer, especially since *B. dissectum* is found when no other species except *B. obliquum* occurs in the vicinity. When we remember that the prothallia are of the tuberous, subterranean, saprophyte type, and not closely associated, the possibility of crossing seems very remote.

We believe the evidence is sufficient to raise a strong presumption that *B. dissectum* is a sterile mutant from *B. obliquum.*

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